

Claims

1. Process for reducing the spontaneous mutation frequencies in a cell or an organism by introducing at least two mutations, whose combined actions lead to at least two enhanced cellular DNA repair mechanisms, into the cell or organism.
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2. Process for producing a cell or an organism with reduced spontaneous mutation frequencies by introducing at least two mutations, whose combined actions lead to at least two enhanced cellular DNA repair mechanisms, into at least one cell of the organism and regenerating the organism therefrom if the organism is a multicellular organism.
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3. Process according to claim 1 or 2, wherein the cell is a prokaryotic cell.
4. Process according to claim 3, wherein the prokaryotic cell is a cell of an archaeobacterium or an eubacterium.
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5. Process according to claim 4, wherein the cell of the eubacterium is a cell of a gram-positive or a gram-negative bacterium.
6. Process according to claim 1 or 2, wherein the cell is a eukaryotic cell.
7. Process according to claim 6, wherein the eukaryotic cell is a fungal cell, animal cell or plant cell.
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8. Process according to any one of claims 1, 2 or 5 to 7, wherein the organism is a fungus, animal or plant.
9. Process according to any one of claims 1 to 8, wherein the combined action of the at least two mutations leads to an enhanced capability of at least two cellular DNA repair mechanism to repair spontaneously occurring mutations.
10. Process according to claim 9, wherein the capability of the mismatch repair system, the post-replicative repair system and/or the SOS repair system to repair spontaneously occurring mutations is enhanced.
11. Process according to any one of claims 1 to 10, wherein the at least two mutations are selected from a mutation leading to an upregulation of the expression of the MutL protein or a homologous protein thereof, a mutation leading to an upregulation of the expression of the MutS protein or a homologous protein thereof, an antimutator allele of a gene encoding DNA polymerase IV or a homologous protein thereof and an antimutator allele of a gene encoding a subunit of DNA polymerase III or a homologous protein thereof.
12. Process according to claim 11, wherein the upregulation of the expression of MutL, MutS or a homologous protein thereof is achieved by introducing a vector within the cell, wherein the vector comprises the *mutL* gene, a gene encoding a homologous protein of MutL, the *mutS* gene or a gene encoding a homologous protein of MutS under the functional control of one or more regulatory units allowing an overexpression of MutL, MutS or a homologous protein thereof.

13. Process according to claim 12, wherein the vector is a multi-copy plasmid.

14. Process according to claim 11 or 12, wherein the regulatory unit is an inducible or constitutive promoter.

5 15. Process according to claim 11, wherein the upregulation of the expression of MutL, MutS or a homologous protein thereof is achieved by introducing one or more additional copies of the respective *mut* gene under the functional control of one or more regulatory units into the chromosome(s) of the host cell and/or by introducing of
10 one or more mutations into that regulatory units controlling the expression of the native Mut Protein such that the production of the respective Mut protein is increased in comparison to a corresponding wild-type cell.

16. Process according to claim 11, wherein the antimutator allele of
15 the gene encoding DNA polymerase IV is *dinB10*.

17. Process according to claim 11, wherein the antimutator allele of the gene encoding the subunit of DNA polymerase III is *dnaE911*.

18. Process according to any one of claims 1 to 17, wherein the combined action of *dinB10* and *dnaE911* reduces the spontaneous
20 mutation frequencies in comparison to a wild-type cell or wild-type organism at least 10-fold.

19. Process according to any one of claims 1 to 18, wherein the combined action of *dinB10*, *dnaE911* and overexpressed *mutL* reduces the spontaneous mutation frequencies in comparison to a
25 wild-type cell or wild-type organism at least 50-fold.

20. Process according to any one of claims 1 to 19, wherein the combined action of the at least two mutations leads to an enhanced cellular viability.
- 5 21. Cell with reduced spontaneous mutation frequencies and/or enhanced cellular viability and obtainable by a process according to any one of claims 1 to 20, wherein the cell comprises at least two mutations, whose combined actions lead to at least two enhanced cellular DNA repair mechanisms.
- 10 22. Cell according to claim 21, wherein the cell is a bacterial, fungal, plant or animal cell.
23. Organism with reduced spontaneous mutation frequencies and obtainable by a process according to any one of claims 1 to 20, wherein the cells of the organism comprise at least two mutations, whose combined actions lead to at least two enhanced cellular DNA
- 15 repair mechanism.
24. *E. coli* MG1655 *dinB10* containing plasmid pmutL (DSM 17016).
25. *E. coli* MG1655 *dinB10 mutL::tet* containing plasmid pmutL (DSM 17017).
26. *E. coli* MG1655 *dnaE zae::cm* containing plasmid pmutL (DSM
- 20 17018).
27. *E. coli* MG1655 *dnaE zae::cm mutL::tet* containing plasmid pmutL (DSM 17019).
28. *E. coli* MG1655 *dinB10 dnaE zae::cm* (DSM 17015).
29. *E. coli* MG1655 *dinB10 dnaE zae::cm mutL::tet* (DSM 17014).

30. *E. coli* MG1655dinB10 *dnaE* *zae::cm* containing plasmid pmutL (DSM 17020).

31. *E. coli* MG1655dinB10 *dnaE* *zae::cm* *mutL::tet* containing plasmid pmutL (DSM 17021).

5 32. Process for the generation of an expression system for a protein wherein the amino acid sequence of the protein is stabilized against spontaneously occurring mutations comprising:

10 a) inserting a nucleic acid sequence encoding the protein into the genome of a host cell, that contains at least two mutations whose combined actions lead to an enhanced capability of at least two cellular DNA repair mechanisms to repair spontaneously occurring mutations, under the functional control of one or more regulatory units allowing an inducible or constitutive expression of the protein, or

15 b) inserting a nucleic acid sequence encoding the protein into a vector under the functional control of one or more regulatory units allowing an inducible or constitutive expression of the protein and transferring the vector into a host cell, that contains at least two mutations whose combined actions lead to an enhanced capability of at least two cellular DNA repair mechanisms to repair spontaneously occurring mutations and

20 c) culturing and/or maintaining the host cell in an appropriate medium.

25 33. Process for the production of a protein wherein the amino acid sequence of the protein is stabilized against spontaneously occurring mutations comprising:

- 5 a) inserting a nucleic acid sequence encoding the protein into the genome of a host cell, that contains at least two mutations whose combined actions lead to an enhanced capability of at least two cellular DNA repair mechanisms to repair spontaneously occurring mutations, under the functional control of one or more regulatory units allowing an inducible or constitutive expression of the protein, or
- 10 b) inserting a nucleic acid sequence encoding the protein into a vector under the functional control of one or more regulatory units allowing an inducible or constitutive expression of the protein and transferring the vector into a host cell, that contains at least two mutations whose combined actions lead to an enhanced capability of at least two cellular DNA repair mechanisms to repair spontaneously occurring mutations,
- 15 c) culturing the host cell in an appropriate medium under conditions allowing the expression of the protein, and
- d) isolating the protein expressed.
34. Process according to claim 33, wherein the protein is isolated from the medium.
- 20 35. Process according to claim 33, wherein the protein is extracted from the host cell.
36. Process according to any one of claims 32 to 35, wherein the protein is a therapeutically usable protein, in particular a cytokine or a growth factor.

37. Process according to any one of claims 32 to 36, wherein the vector is a plasmid, bacteriophage or cosmid.

38. Process according to any one of claims 32 to 37, wherein the regulatory unit is a promoter, a ribosome binding site, an enhancer,
5 a silencer and/or a 3'-transcription terminator.

39. Process according to any one of claims 32 to 38, wherein the nucleic acid sequence encoding the protein is functionally linked to a leader sequence directing the transport of the protein expressed to a cell organelle, a cell compartment, the extracellular space or into the
10 medium.

40. Process for the production of a fermentation product by cultivating a cell producing the fermentation product and/or at least one enzyme involved in the formation of the fermentation product in a medium wherein the genome of the cell is stabilized against spontaneously occurring sequence changes by at least two mutations whose
15 combined actions lead to an enhanced capability of at least two cellular DNA repair mechanisms to repair spontaneously occurring mutations.

41. Process according to claim 40, wherein the fermentation product
20 is a nucleic acid, a nucleoside, a nucleotide, an amino acid, a protein, an acid, a carbohydrate, a vitamin, an antibiotic or an alkaloid.

42. Process according to any one of claims 32 to 41, wherein the capability of the mismatch repair system, the proof-reading function and/or the SOS repair system to repair spontaneously occurring mutations
25 is enhanced.

43. Process according to any one of claims 32 to 42, wherein the at least two mutations are selected from a mutation leading to an upregulation of the expression of the MutL protein or a homologous protein thereof, a mutation leading to an upregulation of the expression of the MutS protein or a homologous protein thereof, an antimutator allele of a gene encoding DNA polymerase IV or a homologous protein thereof and an antimutator allele of a gene encoding a subunit of DNA polymerase III or a homologous protein thereof.
44. Process according to claim 43, wherein the upregulation of the expression of MutL, MutS or a homologous protein thereof is due to the presence a vector within the cell, wherein the vector comprises the *mutL* gene, a gene encoding a homologous protein of MutL, the *mutS* gene or a gene encoding a homologous protein of MutS under the functional control of one or more regulatory units allowing an overexpression of MutL, MutS or the homologous protein thereof.
45. Process according to claim 43, wherein the upregulation of the expression of MutL, MutS or a homologous protein thereof is achieved by introducing one or more additional copies of the respective *mut* gene under the functional control of one or more regulatory units into the chromosome(s) of the host cell and/or by introducing of one or more mutations into that regulatory units controlling the expression of the native Mut Protein such that the production of the respective Mut protein is increased in comparison to a corresponding wild-type cell.
46. Process according to claim 43, wherein the antimutator allele of the gene encoding DNA polymerase IV is *dinB10*.

47. Process according to claim 43, wherein the antimutator allele of the gene encoding the subunit of DNA polymerase III is *dnaE911*.
48. Process according to any one of claims 32 to 47, wherein the combined action of *dinB10* and *dnaE911* reduces the spontaneous mutation frequencies in comparison to a wild-type cell at least 10-fold.
49. Process according to any one of claims 32 to 48, wherein the combined action of *dinB10*, *dnaE911* and overexpressed *mutL* reduces the spontaneous mutation frequencies in comparison to a wild-type cell at least 50-fold.
50. Process according to any one of claims 32 to 49, wherein the combined action of the at least two mutations leads to an enhanced cellular viability.
51. Process according to any one of claims 32 to 50, wherein the cell is a prokaryotic or eukaryotic cell.
52. Process according to claim 51, wherein the cell is a cell of a gram-positive or a gram-negative bacterium.
53. Process according to claim 51, wherein the cell is a fungal cell, animal cell or plant cell.
54. Process according to any one of claims 32 to 53, wherein the cell is a cell according to any one of claims 21, 22 or 24 to 31 or is obtainable by a process according to any one of claims 1 to 20.

55. Process according to any one of claims 32 to 54, wherein the cell is cultivated in a liquid medium.

56. Process according to claim 55, wherein the cell is cultivated in a continuous culture or in a batch culture.

5 57. Process according to any one of claims 32 to 56, wherein the cell is immobilised.

58. Process according to any one of claims 32 to 54, wherein the cell is cultivated on a solid or semi-solid medium.

10 59. Process according to any one of claims 40 to 58, wherein the fermentation product is isolated from the cell.

60. Process according to any one of claims 40 to 58, wherein the fermentation product is isolated from the medium.

61. Protein obtainable by a process according to any one of claims 32 to 60.

15 62. Fermentation product obtainable by a process according to any one of claims 40 to 60.